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Prevalence and distribution of parasites and pathogens of Triatominae from Argentina, with emphasis on *Triatoma infestans* and Triatoma virus TrV

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A B S T R A C T

Chagas' disease is the most important endemic arthropod–zoonosis in Argentina with an estimated 1.6 million people infected with the causative agent *Trypanosoma cruzi*. *Triatoma infestans* is the main vector of Chagas' disease in Argentina. A survey for parasites and pathogens of Triatominae was conducted from August 2002 to February 2005. Collections of insects were made in domiciles, peridomiciles, and in the natural habitats of the Triatominae. Insects from these collections were dissected and their organs and tissues examined for flagellates. Frass from these insects was collected and examined for detection of the entomopathogenic virus Triatoma virus (TrV) using AC-ELISA and PCR. Triatominae belonging to four species, *T. infestans* ($n = 1646$), *Triatoma guasayana* ($n = 4$), *Triatoma platensis* ($n = 1$) and *Triatoma sordida* ($n = 5$) were collected from 62 sites located in 13 provinces of Argentina. Triatoma virus and two protozoan species, *Blastocrithidia triatomae* and *T. cruzi*, the etiological agent of Chagas disease, were found infecting Triatominae. The total prevalence of TrV in 1646 *T. infestans* analyzed by ELISA was 9.66% (159/1646) from 7 to 13 provinces where collections were made. Triatoma virus positive triatomines were found in 17 of 62 populations when examined by AC-ELISA but in 38 of 62 populations when PCR was used for detection. The prevalence of *B. triatomae* in *T. infestans* was 0.43% (7/1646), while the prevalence of *T. cruzi* was 1.3% (21/1646). This is the first study on the diversity, distribution and prevalence of flagellated protozoa and TrV of Triatominae in endemic Chagas' disease regions of Argentina.

1. Introduction

Chagas' is an endemic zoonotic disease in the America's that affects almost 12 million people (OPS, 2006). It is estimated that 1.6 million Argentineans are infected and approximately 15–30% of the cases results in cardiac damage or irreversible lesions in other organs (Segura et al., 2001). Triatomines constitute a very important link in the transmission of *Trypanosoma cruzi* (Chagas, 1909), the etiological agent of Chagas' diseases while *Triatoma infestans* (Klug) is the main vector species of Chagas' diseases in Argentina. Historically, control programs of Triatominae in Argentina have been conducted almost exclusively using synthetic insecticides. Continuous applications of chemical methods for vector control has often been associated with high levels of insecticide resistance and environmental and health concerns. Because of these concerns, alternative vector control is playing an increasingly important role in integrated management strategies.

More than 60 species of Triatominae have been found in nature to be affected by various natural enemies including predators, parasitoids, ecto and endoparasites and pathogens, as well as other poorly defined symbiotic associations (Ryckman and Blankenship, 1984; Weiser, 1991; Coscarón et al., 1999). Parasites and pathogens of Triatominae in Argentina are perhaps the most poorly known of these natural enemies.

Triatoma virus (TrV) is the only entomopathogenic virus found in Triatominae (Marti, 2005). Triatoma virus was identified by Muscio (1988) in *T. infestans* from Córdoba province, Argentina. Subsequently, biochemical, molecular, and crystallographic characterization was reported as well as genomic sequencing (Muscio et al., 1988; Czibener et al., 2000; Rozas-Dennis et al., 2004; Estrozi et al., 2008). This virus is composed of 35% single strand RNA and approximately 10,000 nucleotides and 65% protein. The viral particles are spherical with a diameter of 30 nm and lack an envelope.

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Triatoma virus was included in the Dicistroviridae family, a small group of viruses from insects, with the type species being the cricket paralysis virus (CrPV) (ICTVdB, 2002). Triatoma virus replicates in the midgut epithelium cells of triatomines, causing death of the infected insects (Muscio, 1988). Pathogenicity of the virus and the mechanisms of transmission for TrV have only partially been determined indicating that coprophagy is one of the main routes of infection in laboratory colonies (Muscio et al., 1997, 2000). Vertical transmission of TrV was suspected but not verified (Muscio et al., 1997). There is no information on the distribution or prevalence of TrV in natural populations of triatomines in Argentina.

Several species of protozoa in the genera *Blastocrithidia, Crithidia, Eimeria, Haemogregarina, Hepatozoon, Machadoella, Octosporea, Toxoplasma* and *Trypanosoma* have been found in triatomines, (Ryckman and Blankenship, 1984). The most virulent agents in triatomines are, *Trypanosoma rangeli* (Watkins, 1971; Zeledón and De Monge, 1966) and *Blastocrithidia triatomae* (Schaub, 1988a,b) the later being the Triatominae parasite most intensively studied. *B. triatomae* was described by Cerisola et al. (1971) in *T. infestans* from Córdoba province, Argentina, and different authors studied its ultrastructure (Mehlhorn et al., 1979), mode of transmission (Schaub et al., 1989), pathogenesis (Schaub, 1990a,b), encystment (Reduth and Schaub, 1988) and its culture in vitro (Reduth et al., 1989). In previous studies (Schaub, 1991) mortality rates of 30% were recorded in Triatominae infected with *B. triatomae*. Schaub (1990a) observed clear differences at the cuticle level between infected and healthy *T. infestans*.

Two fungal entomopathogens, *Beauveria bassiana* (Bals) Vuillemin (Marti et al., 2005) and *Paecilomyces lilacinus* (Thom) Samson (Marti et al., 2006) have previously been found infecting Triatominae in Argentina.

The aim of this study was to use classical and modern detection methods to determine the parasites and pathogens (including TrV) of Triatominae in different regions of Argentina. We collected 62 samples during a three years period in order to improve our knowledge on the diversity, prevalence and distribution of flagellated protozoa and TrV of Triatominae in Argentina.

2. Materials and methods

Triatominae were collected from 13 provinces of Argentina (Catamarca, Chaco, Córdoba, La Rioja, Mendoza, Misiones, Neuquén, Salta, San Juan, San Luis, Santa Fe, Santiago del Estero, and Tucumán) from August 2002 to February 2005. Random surveys of Triatoma population were made mainly during the spring and summer months when the greatest increases in the Triatominae species populations occurred. Field collections were made in domiciles, peridomiciles, and a few natural environments. Houses sampled in this study typically had adobe walls, a thatched roof, and two to four rooms or were houses with brick walls and roofs of corrugated metal sheeting. Such a dwelling covered by a single roof defined the domiciliary area. The peridomestic environment consisted of places located within the area of human activity as was defined by Bos (1988) and included a wide array of structures such as sheep, goat, and pig corrals, chicken coops and store rooms. In the wild environment, Triatominae were collected from bird nests and under the bark of trees located more than 300 m from the closest domiciles.

Triatominae were collected individually using metallic forceps; on some occasions in order to facilitate collection of the insects, dislodging substances such as tetramethrin 0.2% were used. Collected insects were transported individually to the laboratory in sterile plastic containers with folded pieces of paper inside and capped with a fine screen. Captured Triatominae were identified according to Lane and Wygodzinsky (1979) and maintained at a temperature of 27 ± 1 °C, 60% ± 5% relative humidity and a photoperiod of 12:12 h (light:dark).

Adults and nymphs (III, IV and V) were observed with a stereomicroscope in order to detect signs and symptoms indicating the presence of pathogens. Symptomatic and asymptomatic insects were dissected as described by Marti (2005) and their organs and tissues examined in fresh wet mounts and Giemsa (10%) stained smears and observed for protozoan infections. Frass samples from each insect were individually placed in Eppendorf tubes and dissolved in 200 μl of phosphate buffed saline (PBS) and kept at −70 °C until used for TrV detection. Detection of TrV infection in frass samples was performed with an antigen-capture enzyme-linked immunosorbent assay (AC-ELISA) and reverse transcription-polymerase chain reaction (RT-PCR) as described by Marti et al. (2008). Briefly, frass samples resuspended in PBS were homogenized in TRIZOL reagent (GIBCO-Invitrogen, USA), and vRNA was purified according to the manufacturer's instructions. For AC-ELISA, hen and rabbit TrV antisera produced in our laboratory were used as capture and detector antibodies, respectively. During 2004 and 2005 all samples were analyzed by AC-ELISA, afterwards, three samples at random from each collection were taken and processed by RT-PCR.

3. Results

A total of 1646 *T. infestans* and 10 insects of three other species of Triatominae, *Triatoma sordida* (Stäl) ($n = 5$), *Triatoma guasayana* (Wygodzinsky and Abalos) ($n = 4$) and *Triatoma platensis* (Neiva) ($n = 1$), were isolated from 62 separate collections made in domiciles (17.4%), peridomiciles (82%), and a few wild environments (0.6%). The RNA virus, Triatoma virus (TrV), and two flagellated protozoans, *B. triatomae* and *T. cruzi* were identified in the Triatominae species examined.

3.1. Triatoma virus

TrV positive tritomines by ELISA were detected in 17 of 62 populations. The range of TrV positive populations varied between 4.54% and 25%, with a single positive specimen of *T. sordida* captured in a house from the Chaco province. TrV infected individuals were found in seven provinces with the greatest levels found in La Rioja province where 5 of 17 populations were positive for TrV (Table 1). When considering the total number of insects examined

Table 1
Natural prevalence of Triatoma virus in Triatominae of Argentina by ELISA.

Province (city)	Date	Habitat	Prevalence (n)
S. del Estero (Jimenez)	December 2003	P	18.18% (4/22)
S. del Estero (Mayusca)	December 2002	D	13.33% (6/45)
San Luis (El Zampal)	April 2003	P	22.82% (76/333)
San Luis (Lomas)	April 2003	P	8.69% (2/23)
Mendoza (San Gabriel)	April 2003	P	9.65% (11/114)
Mendoza (Los Yauyines)	April 2003	P	4.54% (1/22)
Tucumán (Yangallo)	June 2003	P	10% (1/10)
Tucumán (Simoca)	March 2003	P	20% (7/35)
Tucumán (Leales)	December 2002	D	9.52% (2/21)
Chaco (Gral. Guemez)	October 2003	D	100% (1/1[a])
La Rioja (Cuatro Esquinas)	February 2004	P	16.11% (29/180)
La Rioja (La Lomita)	February 2004	P	13.04% (3/23)
La Rioja (Anillaco)	April 2004	P	5.77% (3/52)
La Rioja (Los molinos)	April 2004	P	25% (1/8)
La Rioja (San Blas)	April 2004	P	10% (3/30)
Santa Fe (Los Nocheros)	November 2004	P	11.94% (8/67)
Santa Fe (Villa Mineti)	November 2004	D	25% (1/4)

P = peridomicile, D = domicile.
[a] *Triatoma sordida*, all other triatomines were *T. infestans*.

(1646), TrV was found in 159 triatomines determined by AC-ELISA (9.66%). When the more sensitive method of PCR was used to detect TrV positive triatomines, the number of positive populations increased to 38 of 62 examined from eight provinces (Fig. 1).

3.2. *B. triatomae*

This flagellate was exclusively found in the midgut lumen of *T. infestans*. Flagellates were easily recognized in wet mount preparations of the intestinal content by the presence of sphero, pro, and epimastigote stages free in the gut lumen when viewed under a phase contrast microscope. In addition, epimastigotes were frequently observed with several resistant cysts, usually called "straphanger", attached close to the base of the flagellum (Fig. 2). The presence of *B. triatomae* in triatomines was detected in 3 out of 62 samples. Triatomines infected with *B. triatomae* were collected in localities of two provinces Tucumán (in March 2003) and one of Santiago del Estero (in December 2003) (Fig. 1). The range of *B. triatomae* in positive populations of *T. infestans* varied between 4.16%, 5.71% and 37.5%, respectively. The total prevalence of *B. triatomae* in the midguts of the examined insects was 0.42% (n = 7/1646).

3.3. *T. cruzi*

This flagellate was exclusively found in *T. infestans* during this study. Epi, and trypomastigote forms of this parasite were the stages most commonly observed in the midgut content of dissected *T. infestans* in fresh preparations observed under phase contrast microscopy. *T. cruzi* was found in 9 of the 62 sampled *T. infestans* populations from three provinces, Salta, Santiago del Estero, and Tucumán (Fig. 1). *T. cruzi* was most common in *T. infestans* from Santiago del Estero province where 6 of 9 populations were positive. The range of *T. cruzi* infections in positive populations of *T. infestans* varied between 5.55% and 42.8% (Table 2). The total prevalence of *T. cruzi* in field collected *T. infestans* was 1.27% (21/1646).

4. Discussion

This survey represents the largest study conducted on the natural occurrence of parasites and pathogens in Triatominae vectors of Chagas' disease in the Neotropical region. Pathogen diversity was low in this three years study. Here were report the presence of *B. triatomae*, *T. cruzi*, and the RNA virus, Triatoma virus (TrV).

The ELISA capture method for the diagnosis of TrV in frass samples was very sensitive for detection of this virus in field collected triatomines. When utilizing AC-ELISA as the screening method, TrV had an overall prevalence of 9.66% and was found in 17 Triatominae populations from 7 of 13 provinces. The PCR detection method had higher sensitivity than AC-ELISA increasing the number of provinces with infected individuals to eight. For the collected triatomines, 17.5% were captured in domiciles and the rest in peridomiciles, with almost all the insects infected with TrV captured in peridomicile habitats. However, based on Table 1, while the sample sizes were dissimilar, the overall percentage infection of TrV from insects collected in domiciles and peridomiciles were not different (10 of 71 insects from domiciles (14.1%) and 149 of 919 from peridomiciles (16.2%) were infected).

The only TrV infected specimen of *T. sordida*, was captured in a domicile in Chaco province that is not usual to this wild specie (Carcavalho et al., 1998). *T. sordida* is a new host for TrV, increasing

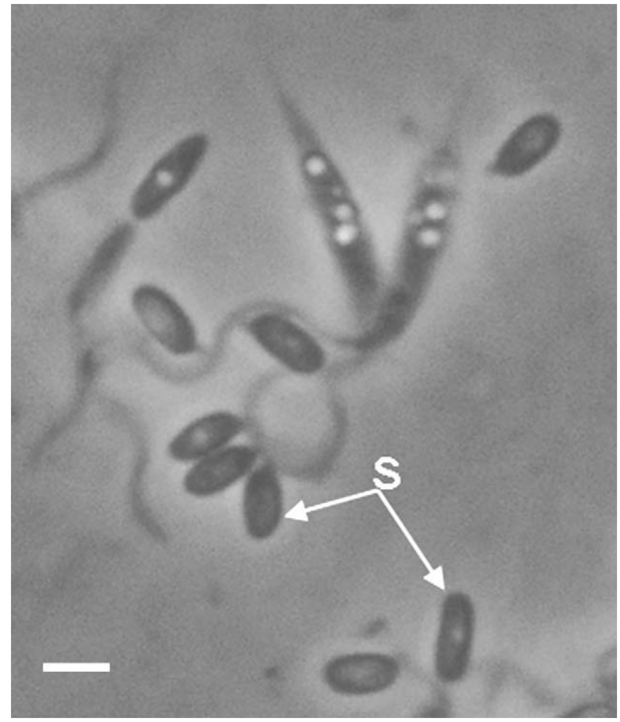

Fig. 2. Epimastigote stages of *Blastocrithidia triatomae* free in the gut lumen of *Triatoma infestans* with "straphanger"(s) attached close to the base of the flagellum (phase contrast microscopy). Bar = 4 µm.

Table 2
Prevalence of *Trypanosoma cruzi* in *Triatoma infestans* of Argentina.

Province (city)	Date	Habitat	Prevalence (n)
Tucumán (Leales)	December 2002	P	42.8% (9/21)
Tucumán (Los Chañaritos)	June 2003	P	5.88% (1/17)
S. del Estero (Río Hondo)	June 2003	P	11.11% (1/9)
S. del Estero (Tucu Yacu)	June 2003	P	21.05% (4/19)
S. del Estero (El Bagual)	June 2003	P	12.5% (1/8)
S. del Estero (Bobadal)	December 2003	P	7.69% (1/13)
S. del Estero (Isca Yacú)	December 2003	P	7.4% (2/27)
S. del Estero (El Charco)	December 2003	P	33.33% (1/3)
Salta (Obraje)	December 2004	P	5.55% (1/18)

P = peridomicile.

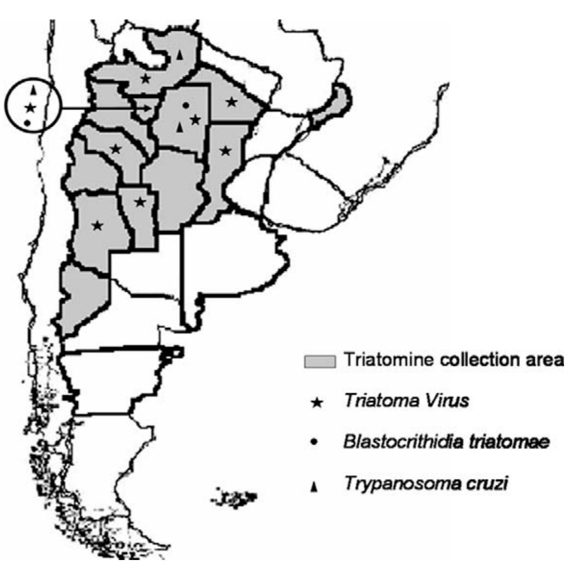

Fig. 1. Geographical distribution of *Blastocrithidia triatomae*, *Trypanosoma cruzi* and TrV in natural populations of Triatominae in Argentina.

the number of susceptible species to nine; *Rodnius prolixus*, *T. delpontei, T. infestans, T. pallidipennis, T. platensis*, and *T. rubrovaria* by viral intrahemocelic injection (Muscio, 1988), and by *per os* ingestion of viral particles in *T. infestans* (Muscio, 1988; Rozas-Dennis and Cazzaniga, 2000), *T. patagonica* (Rozas-Dennis et al., 2002), and *T. guasayana* (Rozas-Dennis and Cazzaniga, 1997).

Schaub (1990a) reported that *B. triatomae* infected *T. infestans* are characterized by having a soft, pale brown cuticle when compared with healthy *B. triatomae* insects which have a harder, dark brown cuticle. Four of the seven infected *T. infestans* found expressed those signs of infection but the other three insects did not and appeared similar to the uninfected *T. infestans*. In addition, several other field collected *T. infestans* had a pale brown cuticle but were not infected with *B. triatomae*. The presence of cysts, "straphangers", attached to the flagellum of the epimastigotes was the diagnostic characteristic used to identify *B. triatomae* instead of the presence of vacuoles in the cytoplasm as indicated by Mehlhorn et al. (1979).

Ecological data for *B. triatomae* in nature are very limited. Cerisola et al. (1971) made the first isolation of *B. triatomae* from *T. infestans* in Córdoba province, Argentina. Cecere et al. (2003) did not report the presence of *B. triatomae* or *T. cruzi* in field collected Triatominae from Córdoba province during a two year study. Here we have extended the geographic distribution of this flagellate to two other provinces of north central (Santiago del Estero) and northwest (Tucumán) Argentina.

The low prevalence of *B. triatomae* recorded in our survey was unexpected. The presence of resistance cysts, horizontal transmission by coprophagy and the relative low pathogenicity of this flagellate to their host are usually characteristics that would indicate a more widely distributed parasites in nature.

Argentina Public Health Ministry has divided the country into four groups of provinces according to the risk of acute Chagas' disease by vector transmission (Zaidemberg et al., 2004). Group 1 contains provinces where vectorial transmission has been interrupted by control measures (Jujuy, La Pampa, Neuquen, and Río Negro); Group 2 contains provinces where vectorial transmission is suspected to be interrupted but not confirmed (Catamarca, Entre Ríos, Salta, Santa Fe and Tucumán); Group 3 contains provinces with confirmed vectorial transmission and occurrence of acute cases of Chagas' disease (Chaco, Córdoba, Formosa, La Rioja, San Juan, and Santiago del Estero); Group 4 contains provinces for which information on Chagas vectorial transmission is limited (Corrientes, Mendoza, Misiones, and San Luis) (Zaidemberg et al., 2004). We found *T. infestans* infected with *T. cruzi* in nine localities of Argentina with six located in Santiago del Estero, a province belonging to group 3 (higher risk for acute Chagas' disease). The other three localities with infected *T. infestans*, two in Tucumán and one in Salta province, belong to the second most risky area (Group 2) where vector transmission is suspected. Infected insects were recorded in nine *T. infestans* populations collected exclusively during December (5) and June (4) during the 3 year survey.

During this 3 year study, we found a relatively low diversity of parasites and pathogens in Triatominae from Argentina. Apparently, host blood ingestion does not facilitate entomopathogen transmission and/or infection. Jenkins (1964), Roberts and Strand (1977), Roberts (1980) and Daoust (1983), reported a significant difference in the number of entomopathogens from strictly hematophagous insects such as Reduviidae, Cimicidae (Hemiptera) and lice (Phtyraptera), when compared with the number of pathogens recorded from other hematophagous insects during the adult stage such as Culicidae, Simuliidae, Glossinidae (Diptera). For instance, Jenkins (1964) cited 16 parasites and pathogens in Reduviidae and 212, 45, and 70 pathogens in Culicidae, Glossinidae, and Simuliidae, respectively, with the majority of these recorded from the larval stages (Weiser, 1991). Differences are even larger if entomopathogen diversity of Reduviidae is compared with chewing insects such as Coleoptera, Lepidoptera and Orthoptera. We speculate that low diversity of pathogens could be caused, in part, as a result of the obligatory hematophagy of Triatominae which makes horizontal transmission of entomopathogen difficult with coprophagy the principal mechanism for pathogen transmission.

The number of Triatominae species in the Americas varies from 110 to 116, according to the authors Moreno and Carcavalho, 1999. Almost all the Triatominae captured and examined in this study were collected in domicile and peridomicile habitats. The low Triatominae diversity that characterizes these habitats may be an important factor that explains the low pathogen diversity recorded. We speculate that the larger Triatominae species diversity reported in a very large spectrum of wild habitats, more than 80 species (Carcavalho et al., 1998), could be important for the maintenance of natural enemies in Triatominae and is an area that requires additional investigation.

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